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Comparison of Thiocyanate Levels in Tobacco Users: A Cross Sectional Study

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ABSTRACT

Background: Saliva, composed of over 99% water, serves as a diagnostic fluid for conditions like diabetes, rheumatoid arthritis, and hepatitis. It is easily accessible and non-invasive. Tobacco smoke contains harmful gases, including cyanide, which metabolizes into thiocyanate (SCN−), a key biomarker of smoke inhalation linked to neurological damage, delayed wound healing, and periodontal disease.
Objectives: To investigate and compare thiocyanate as a biochemical marker in smokeless tobacco user, smoked tobacco users and non-tobacco users.
Materials and Methods: Following standard protocols five (5) ml saliva was collected by spit method technique as per Navazesh et al from 234 subjects. Supernatants of saliva were achieved by centrifuging collected saliva at 3000 rpm for 10 minutes. For evaluation of salivary SCN level, 0.5ml of salivary supernatant was dissolved in 5 ml of Ferric nitrate (Fe [NO3]3) forming dark red color of the mix in presence of thiocyanate. Ultra-violet spectrophotometry of solution was done at 447 nm wavelength. Data were checked for its normality, ANOVA was applied for significance amongst various groups and T-test was applied for comparison between groups.
Results: Mean salivary thiocyanate levels amongst Non-tobacco users, Smokeless tobacco users and Smoked tobacco users were 1.92 mM, 2.25 mM and 2.76 mM respectively. Smoked tobacco users had the highest thiocyanate levels, followed by smokeless tobacco users, with a statistically significant difference (p = 0.043).
Conclusion: Salivary thiocyanate levels were significantly higher in tobacco users than non-users, with the highest levels in smoked tobacco users.
Keywords: Antioxidant, Saliva, Smokeless Tobacco, Thiocyanate.

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INTRODUCTION

Human saliva is a complex biological fluid produced by major and minor salivary glands, with an average daily production of approximately 750 mL. As the first bodily fluid to interact with food, beverages, and inhaled substances, saliva plays a crucial role in oral health. Composed of over 99% water, it forms a protective seromucous fluid that lubricates oral tissues and serves as a barrier against irritants, including hydrolytic enzymes in plaque, carcinogens from smoking, and other exogenous chemicals.[2] Given the oral cavity's role as both an entry and exit point of the body, saliva has significant diagnostic potential, particularly in the early detection of diseases. It has been explored as a noninvasive diagnostic tool for conditions such as diabetes, rheumatoid arthritis, celiac disease, and hepatitis, offering a more convenient alternative to blood and urine sampling.[3] Saliva's immunological and nonimmunological defense mechanisms rely on proteins and secretory Immunoglobulin A (IgA).[4] Additionally, its composition includes thiocyanates, uric acid, ascorbic acid, albumin, agglutinin, lactoferrin, glutathione, lysozymes, and trace amounts of transferrin, all of which are influenced by physiological factors, personal habits, and diet.[5]

Tobacco smoke contains a variety of gases, primarily carbon monoxide and hydrogen cyanide.[6,4] Cyanide metabolism leads to the formation of thiocyanate (SCN−), a key biomarker of smoke inhalation alongside carbon monoxide, nicotine, and cotinine. Thiocyanate, a detoxification product of cyanide, is produced endogenously through dietary sources, particularly cruciferous vegetables, and can also result from certain medications used to treat hypertension and thyroid disorders.[7,8] It is present in bodily fluids such as breast milk, tears, and cow's milk, and its presence is significantly elevated in tobacco users due to cyanide exposure. With a half-life of approximately 10–14 days, SCN− serves as a reliable biomarker for confirming tobacco use.[7] Elevated thiocyanate levels indicate chronic cyanide exposure, which is associated with adverse effects such as neurological disturbances, delayed wound healing, and periodontal disease.[7]

Tobacco users, whether consuming smoked or smokeless forms, often experience delayed healing following tooth extractions, periodontal procedures, and implant surgeries. One contributing factor may be elevated salivary SCN− levels. This study aims to determinrker among smokers, smokeless tobacco users, and non-tobacco users. The findings will provide valuable insights for dental care providers and policymakers, potentially leading to new regulations that reduce the burden on national dental health systems.

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MATERIALS AND METHODS

This cross-sectional study was initiated from 26th November 2020 completed on 13th May 2021. Non-probability convenience sampling was used to select all eligible subjects. Saliva of eligible subjects was collected from outpatient department of Sardar Begum Dental Hospital, Gandhara University, Peshawar and the procedure was carried out in the Department of Pharmacy, University of Peshawar, Khyber Pakhtunkhwa, Pakistan. Ethical approval for the study was achieved in 23rd ethical committee of Gandhara University held on 12th November 2020.

Study population consist of male participants age between 20-60 years, reported at Outpatient department of Sardar Begum Dental Hospital, Gandhara University, Peshawar, Khyber Pukhtunkhwa during the period of data collection. Female and young participants and were excluded due to cultural norms as the local feminine and young population try to avoid tobacco use in public and hesitate to share such information. According to Charan et al. WHO formula was used to calculate sample size, calculated sample size 234 (77 in each group 'r') was estimated by 95% confidence level.[8]

Systemic Diseases like diabetes, chronic liver disease, hypertension, Cardiovascular disease, kidney disease, chronic lung disease and thyroid problem, drug users, malignancies were excluded. After approval from hospital ethical committee (23rd ethical meeting 12th Nov 2020), a total of 234 subjects were enrolled which fulfilled the criteria of inclusion in this study. The aim, procedure, risk and benefits of the study were explained to the enrolled individuals. In this study the data was collected on a structured and printed proforma. An informed consent was taken from selected individuals prior to the procedure. The enrolled individuals were divided into tobacco users and non-tobacco users. Each group had 78 individuals, confidentiality of their personal data was assured. The data comprised of name, age, average number of tobacco smoked daily, tobacco chewing daily, monthly income, monthly expense, past medical history and thiocyanate levels.

To ensure quality control each subject was instructed to avoid drinks, food specially vegetables cabbage family, caffeine and tobacco consumption two hours before saliva collection. Five mL saliva was collected from each subject by spitting method according to Navazesh et al,[9] subjects were instructed for rinsing their mouth with water to remove any debris 10 minutes prior to saliva collection. The rinsing procedure was first demonstrated to each subject. Instructions were also given not to brush teeth within 60 minutes of sample collection. Each subject was given a 10 ml graduated test tube and was instructed to collect saliva for five minutes and spit it in the test tube following this protocol five ml saliva was collected. Collected saliva having blood was discarded. Supernatants of saliva was achieved by centrifuging the collected saliva immediately at 3000 rpm for 10 minutes in centrifuge (Fisher Scientific, USA, model 800, serial no. 92039, inspection no. 09, manufacturing date 10/2004).

The Stock solution was prepared for determination of thiocyanate. The first stock solution was made in a volumetric flask, mixed with 0.2M $Fe(NO_3)_3$ of 1M HNO_3 and the second stock solution was mixed with aqueous solution of 2.00×10^{-4} M KSCN. To check the nature of solution, 1 ml of saliva was collected in test tube and added 1 ml of ferric nitrate solution, the resulting complex of ferric thiocyanate ($FeSCN^{+2}$) formed deep red color (Fig. 01). The solutions of different concentrations were prepared in a test tube for the determination of molar absorption of $FeSCN^{+2}$ complex. The solution was started from zero concentration of thiocyanate and steadily increased with decreasing amount of distilled water. Ferric nitrate volume was same in all test tubes. The absorbance of every test tube solution was checked using spectrophotometer on 447nm wavelength.

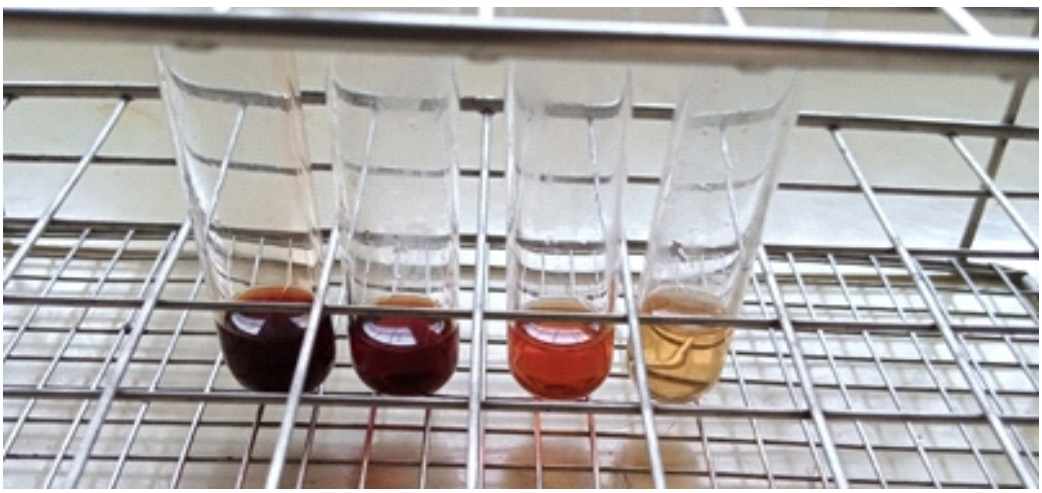


Figure 01. Showing deep red colour after mixing saliva supernatant with ferric thiocyanate

Data Analysis

The data were compiled as well as analyzed in SPSS version 20. Descriptive statistics were used to describe, organize and summarize data. Data was normally distributed using shapiro wilk test. All results were presented in the form of tables and graphs. One-way ANOVA and student T test were applied for checking significance amongst Smokeless tobacco users, smoked tobacco users and non- tobacco users. Whereas level significance was kept < 0.05.

RESULTS

The sample size for the study was 234 which was calce and compare salivary thiocyanate levels as a biochemical maulated with an estimated by confidence level of 95% with 80% power of test and 0.8 as margin of error (Statistix 8.1). Subjects included in the current study had an age range of 20-60 years. Non-smokers are considered as control group for the study, which was compared with that of subjects consuming smoked tobacco and smokeless tobacco. The mean age in Non-smokers Group was 36 ± 2 years while in Smoked Tobacco Group and Smokeless Tobacco Group were 38 ± 2 years and 39 ± 1.5 years, respectively. The mean values calculated as a result of photo spectrometry for "Non- tobacco users, smokeless tobacco users and smoked tobacco users" were 1.9155775, 2.2549227 and 2.7567363 respectively.

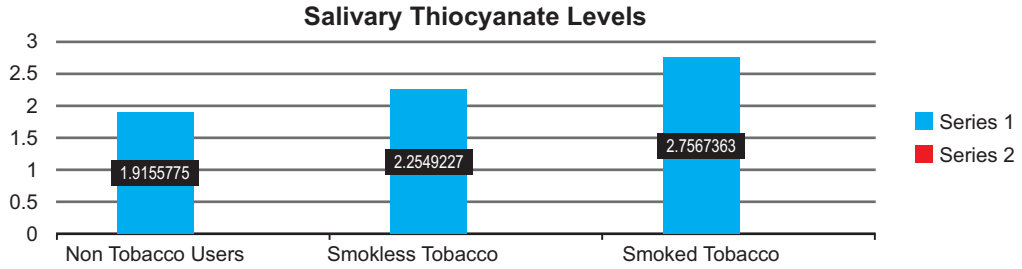


Figure 02. Graph showing means of Non-tobacco users, Smoked tobacco users and Smokeless tobacco users.

In the present study, the analysis of salivary thiocyanate level showed that the maximum level was detected in tobacco smokers 2.7567363 mM and smokeless tobacco 2.2549227mM than non-tobacco users 1.9155775mM. The difference in salivary thiocyanate levels between the tobacco users and non- tobacco users displayed statistically significant results p = 0.043.

This ANOVA results demonstrates the effect of the independent variable, non-tobacco users, on a dependent variable. The total variance in the data is 1.324, of which 0.070 is explained by the independent variable, while 1.254 remains unexplained as residual variance. With one degree of freedom for the regression and 76 for the residual, the mean squares are 0.070 and 0.016, respectively. The F-statistic, calculated as 4.251, measures the strength of the independent variable's effect. The significance value (p = 0.043) indicates that this effect is statistically significant, as it falls below the conventional threshold of 0.05. This suggests that there is a meaningful difference between groups, and non-tobacco use has a significant influence on the dependent variable, though the explained variance is relatively small.

Table 01. ANOVA showing the significance level of the study

	Sum of Sqaures	Df	Mean Square	F	Sig.
Regression	.070	1	.070	4.251	.043
Residual	1.254	76	.016		
Total	1.324	77			

The independent variable is non-tobacco users.

The data collected was computed for Mean, standard deviation and standard error using a One- Sample test. Amongst these the smoked tobacco users showed the highest mean value while the non-tobacco users showed the least mean value for likelihood. (Table 02)

Table 02. One sample t-test showing means and standard deviations

	N	Means	Stand. Dev	Std. Error Means
Smoked Tobacco	78	2.7567363	.22785339	.02579932
Smokeless Tobacco	78	2.2549227	.13112250	.01484670
Non-Tobacco users	78	1.9155775	.11909496	.01348485

The independent variable is non-tobacco users.

DISCUSSION

This research aimed primarily to determine whether tobacco smokers possess higher thiocyanate amounts than non-smokers do. The study found that smokers presented higher thiocyanate concentrations than non-smokers while showing that smoked tobacco users had 2.75 ± 0.22 mean thiocyanate levels and smokeless tobacco users had 2.25 ± 0.13 and non-tobacco users had 1.91 ± 0.11. Researchers have already reported in previous studies that smokers in Karnataka and Mangalore, India exhibited higher thiocyanate levels than passive smokers and users of smokeless tobacco and non-tobacco consumers ($P \leq 0.0001$). Salivary thiocyanate became an accepted diagnostic biomarker after researchers found it workable for differentiating tobacco users from non-users.[10]

An Indian research study on periodontally healthy patients, smokers, and gutka chewers showed higher salivary thiocyanate levels in chronic periodontitis subjects and gutka chewers as well as smokers.[11] These results support the findings of our study which confirms tobacco use elevates salivary thiocyanate levels. A research investigation by Kumar C. N. et al. determined that smokers with chronic periodontitis possessed elevated salivary thiocyanate levels than non-smoking patients with chronic periodontitis.[12]

Different research findings diverge from each other regarding their results. Another research investigation demonstrated that smokers did not have different thiocyanate concentrations than non-smokers and detected higher concentrations in non-tobacco users. Non-smokers presented significantly elevated thiocyanate levels when compared to other groups according to this study. Results indicated 121.25–187.54 mg/L in tobacco smokers while e-cigarette smokers had 121.24–244.11 mg/L and non-smokers had 33.03–79.49 mg/L respectively ($P < 0.05$). The contradiction exists due to differences in research sample dimensions and methodology along with population demographic features. The research results could have been impacted by e-cigarette user participation since these users might exhibit different thiocyanate metabolic patterns. Studies that evaluate thiocyanate levels through exposure to this substance from electroplating or metal refining and consumption of cabbage and garlic might account for the observed disparities across research conclusions.[12,13]

Fat factors and the environment contribute significantly to the amounts of thiocyanate present in the body. A large body of research indicates that dietary thiocyanate exists at greater levels in vegetarians but their concentrations never exceed those of tobacco consumers. The researchers emphasized that dietary products and environmental elements impact thiocyanate amounts however smokers demonstrate the highest levels. Mainstream tobacco smoke delivers 40–70 ppm hydrogen cyanide to the lung while sidestream smoke delivers less than 5 ppm thus explaining why smokers have higher thiocyanate concentrations than passive smokers and non-smokers.[14]

Expert research should address the connection between thiocyanate measurements and financial standing. The research demonstrated that tobacco users received greater monthly income together with enhanced monthly spending yet failed to establish any economic relationship to tobacco consumption. Studies demonstrate that individuals with low socioeconomic status show strong tendencies to persist in smoking habits especially among developing nation adult males. The combination of financial strain alongside inadequate healthcare access and unemployment leads people to start smoking which serves as their way to cope with difficult situations. Unemployed adults together with teenagers often face heightened tobacco consumption levels because their financial situations and increased stress result in this behavior.[15]

The research faces potential limitations due to its self-reporting method of smoking status because this technique can produce biased information. Future research needs to add biochemical testing techniques based on cotinine measurement to boost the study precision. The analysis of thiocyanate levels requires larger sample groups of diverse demographics to validate environmental and occupational factors and dietary patterns. Research into these influencing factors will enable a better application of thiocyanates as biomarkers for tobacco exposure assessment and their effects on public health concerns.

CONCLUSION

Within the limitations of this study, it may be concluded that.

- Individuals usings smoked tobacco and smokeless tobacco had significantly high levels of thiocyanate as compared to non-tobacco users.
- Smoked tobacco users have high level of thiocyanate as compared to those who use smokeless tobacco.

Recommendations

In the light of current findings it is recommended that

- Further study should be carried out on larger population to know tobacco using in both genders.
- Salivary SCN should be assessed in Pan & gutka chewers.
- Saliva should be analyzed for other ingredients that may be responsible for causing oxidative stress.

Ethical Consideration

This study was approved by Institutional Review Board of Sardar Begum Dental Hospital, Gandhara University, Peshawar, Pakistan.

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Conflict of Interest

Authors declared no conflict of interest.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

AUTHORS' CONTRIBUTION

The following authors fulfill authorship criteria as per ICMJE guidelines;

Khan JN, Shah MA: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Nausheen N, Nisar P: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

Siddique HMAB: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.

Tanzeela: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.

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